Art. XXI.—*Notes on Blood Parasites.*

BY

J. A. GILRUTH, D.V.Sc., M.R.C.V.S., F.R.S.E.,

GEORGINA SWEET, D.Sc.

AND

S. DODD, D.V.Sc., F.R.C.V.S.

(With Plates XXVIII.-XXX.)

[Read 14th July, 1910.]

During the past few weeks several interesting Haematozoa have come under our notice, three of which are herein recorded. The specimens of host animals in which these parasites were found were sent to the Veterinary Research Institute, for post mortem examination, from the Zoological Gardens of Melbourne.

I.—Proteosoma biziurae, n. sp.

(Plate XXVIII., Figs. 1–15).

Host animal, *Biziura lobata* (Musk Duck).

So far as we are aware, the only records of Plasmodidae from Australian birds are *Plasmodium praecox (?)* from sparrow (Johnston, 1909, p. 581), afterwards described as *Plasmodium passeris*, Jnstn., by Cleland and Johnston (1909, p. 505).

As regards the generic name adopted for this parasite, we have decided, following Doflein (p. 657) to retain the original generic name *Proteosoma* (Labbé, 1894, p. 157) for those melanin-producing plasmodia of Birds having more or less irregular, round or pear-shaped gametocytes, which when mature distort the erythrocyte and displace the nucleus. On these grounds, *Plasmodium passeris*, Jnstn., should be rather *Proteosoma passeris.* The application of a new specific name to this parasite appears to us to be justified by its apparent dissimi-

larity from other species of this genus, apart from the point that it seems to be the only plasmodial form recorded from the order Anseriformes of Birds.

These parasites were found in two specimens of Musk Duck obtained at different times. Very few cells were affected, so that often none could be seen in many consecutive "fields." The size of the normal erythrocyte is 13.5 to 14.2 μ long by 7.8 to 8.5 μ wide. The parasite varies in size from 8.5 to 10 μ long by 4.8 to 9.7 μ wide in apparently fully-developed forms, thus causing an increase in size and a distortion of the infected erythrocytes, which may reach 15.6 μ long and 9.7 to 10.6 μ wide in size, and become irregular, pyriform, polyhedral, or spherical in shape.

Both young forms (cf. Figs. 7, 8 and 12) and mature gametocytes were found (cf. Figs. 3, 9, 13, 14 and 15). In the young parasites which are elongated, e.g., Fig. 7, no nucleus could be seen, but numerous fine granules of pigment are present. Later, as in Fig 8, large melanin-pigment granules appear, this figure also illustrating their occasional triangular form. In the one shown in Fig. 12, a small well-defined vesicular nucleus can be seen. The later stages show a gradual approach to the spherical form found in the mature parasite, e.g., Figs. 3, 9, 13, 14, 15. With the increasing size and more spherical form of the parasite, the nucleus of the host-cell becomes displaced from the centre and pushed more or less to one end or side. In the former case, the direction of the long axis of the nucleus is changed, and it comes to lie obliquely across the length of the erythrocyte much in the same way as happens in the host-cells of *Proteosoma praecox*. No bean-shaped or gregarinoid forms similar to those in *P. praecox* could be found, unless the form shown in Fig. 6 may be so regarded. The spherical shape of the gametocytes reminds one of those of *P. passeris*, but the parasites of the Musk Duck are larger than those of the sparrow, being really intermediate in size between *P. vaughani* and *P. passeris* 5 to 6.5 μ), and P. majoris (11 to 12 μ).

Both male and female gametocytes are present, but no sporulation stages could be found. The male gametocytes are represented by Figs. 14 and 15, in which the body of the

parasite, which is oval, is more hyaline, the nucleus very diffuse, and the melanin pigment granules disposed at either pole.

The fully-developed female gametocytes (Figs. 3, 9 and 13) show a spherical outline, a more granular body, a more or less general distribution of the large melanin granules and a more definite clear nucleus, except in Fig. 3, in which no nucleus could be detected at all. No erythrocytes could be found in which the parasite had occasioned the loss of the host nucleus; nor were any free forms to be found except that in Fig. 10, in which the body was hyaline with a faintly staining diffuse nucleus near the broader end, and the partly free much more coarsely granular form in Fig. 5. Five typical forms may be described as follows:—

1. The nucleus of the red blood corpuscle is pushed close to the edge, there being little cytoplasm in the host-cell, the greater portion of which is occupied by the parasite. The latter has a very slightly eccentric homogeneous nucleus staining pink (with Giemsa's stain), the finely granular protoplasm staining a deep blue. The body contains irregular but evenly distributed fine pigment granules. No limiting membrane was present (cf. Fig. 9).

2. The nucleus of the host-cell which contains a large amount of protoplasm is pushed to one side. The parasite has a large pink-stained nucleus almost filling the body with faint blue protoplasm at either side, containing fine granules (cf. Fig. 15).

3. Host-cell is not always enlarged, but the nucleus is pushed to one side; the parasite is very faint and pinkish throughout. Large granules are distributed more or less generally. No distinct nucleus is to be seen. Definite limiting membrane is present (cf. Fig. 4).

In such as shown in Fig. 6, two vesicular nuclei are seen within a very granular protoplasmic body.

4. The host-cell is enlarged and somewhat pyriform. The nucleus is slightly displaced by the oval parasite, which has a diffuse pinkish sometimes eccentric nucleus, in a bluish body, more deeply stained at one extremity. The pigment granules are irregular, but more or less polar in distribution (cf. Fig. 14).

5. The host-cell is more markedly pyriform, and the nucleus much displaced. The parasite has a spherical pinkish finely granular body with no defined nucleus and coarse granules scattered throughout (cf. Fig. 3. Fig. 13 is similar, but shows a slightly defined, small, eccentric nucleus in the parasite, which occupies a more oval host-cell).

2.—Haemogregarina megalocystis, n. sp.

(Plate XXIX., Figs. 1–12).

Host animal *Python spilotes*, var. *variegata*.

As will be seen this reptilian parasite is to be regarded as belonging to the genus *Haemogregarina* (s.s.) (cf. Doflein p. 681-2) rather than *Karyolysus* since the atrophied nucleus associated with the presence of the latter is absent here, although a degeneration of the stroma of the host erythrocyte is present somewhat comparable to that found in the case of *Karyolysus lacertarum*.

The blood smears taken from this host showed very many of the red blood corpuscles to be affected with haemogregarines, two or three in one "field" not being uncommonly seen, while in one chance "field" 1.1 mm. in diameter, 10 such infected erythrocytes were counted to about 600 normal corpuscles.

The infected host-cells are always enlarged, being generally two to three times the normal size, though so far as we could find not more than one parasite is present in each. The cytoplasm of the host-cell is extremely tenuous, and in the larger forms is completely dehaemoglobinised, so much so that when overlapping another corpuscle little or no obscuration can be detected. In the less enlarged host-cells, the dehaemoglobinisation is proportionately less noticeable, the staining in the former case showing very faintly purple, against the distinct red of the normal erythrocyte. The nucleus of the host-cell is distinct often displaced towards one extremity, or to one side, and rarely lying on the convex borders of the parasite. The influence of the parasite on the host-nucleus is seen not in atrophy, but in its longer, and rarely narrower, sometimes wider outline, while it stains much more deeply and more

homogeneously than do the unaffected nuclei. The size of the normal erythrocyte varies from 15.2 μ long by 3.7 μ wide to rarely 21.3 μ long by 4 μ wide. That of the infected cells varies from 22 μ long by 14.2 μ wide up to 60 μ long by 28 μ wide, 35 to 55 μ being the more frequent lengths by about 20 μ in width. The parasite itself lies in the central region of the host-cell alongside the host-nucleus. It is gregarinoid in form. In size it nearly always exceeds that of the host nucleus, and often that of the majority of the normal erythrocytes. Thus it varies from 13 μ long by 4 μ wide up to 18.6 μ long by 5.6 μ wide, 14 to 16 μ in length by 5 μ in width being the more frequent. All forms seen were sporonts, no forms showing the division stages of the sexual cycle being found. The body is fairly homogeneous, somewhat granular and blue (with Giemsa smears) often fainter at one extremity than elsewhere. The nucleus is generally spherical, pinkish with deeper stained almost reddish granules, sometimes distributed chiefly in a radial manner at the periphery, but often fairly regularly throughout the nucleus. It may be central, but at times lies towards one extremity. The capsule of the parasite is rarely seen (cf. Figs. 3, 4 and 10), but often the whole parasite, whether capsule is present or not, is seen surrounded by a faint zone, limited by a definite thin line, which appears to enclose the nucleus of the host-cell as well as the parasite. Doubtless this area represents an area of degenerated stroma similar to that shown in the case of *Karyolysus lacertarum*, though in this case no granulation is observable. The curious appearance of the host nucleus shown in Fig. 7, a blue-stained, kidney-shaped mass surrounding a pink homogeneous centre, together with the degeneration of the stroma, in other forms is certainly suggestive of an affinity with *Karyolysus*. The hooked tail of the homogregarinoid form is well seen in Fig. 11, in which also the host-cell nucleus is markedly enlarged and altered in shape. In rare cases enlarged cells were found, such as shown in Figs. 9a and 9b, in which no parasite was visible. It is improbable that these are cells which have been vacated by a parasite, since they are not fully enlarged, and are normal except in size and in the enlarged nucleus. Fig 8 shows a curious appearance found in

a leucocyte, in which a deeply-stained, rosette-like mass of chromatin (?) was present close beside the normal leucocyte nucleus. The condition of the host-cells here is somewhat comparable to that found by Sambon (1907 p. 284) in *Coluber corais* var. *couperi*, though he does not mention any such degeneration of the host stroma; and further the bean-shaped forms of *H. rarefaciens*, Sambon, are considerably smaller than many of those met with here. Moreover the parasite in *H. megalocystis* (n.sp.), so far as we could find, does not show any of the long narrow forms found in *H. rarefaciens*, and unless Fig. 8 represents some stage of the former, we could find no parasites in the leucocytes of this specimen of *Python spilotes* var. *variegata*. In view of these differences from Sambon's form, and of the different families of the hosts, we feel justified in the meantime in regarding this as a new species.

The blood of this specimen shows large numbers of short bacilli, with distinct capsule, often in long chains, and sometimes sporulating; they were isolated, and grow readily in ordinary media.

3.—Microfilaria gymnorhinae, n. sp.

(Plate XXX., Figs. 1–3).

Host animal—*Gymnorhina tibicen.*

Up to the present no blood-filariae appear to have been found in the birds of Southern Australia, though adults of *Filaria tricuspis*, Fedtsch, have been recorded from 3 birds belonging to the Bismarck Archipelago to the North of Australia (Von Linstow, 1897, p. 283), of *Filaria flabellata*, Von Linst., and *Filaria paradisea*, Von Linst., from *Paradisea apoda* from the Aru Is., also lying to the North of Australia (Von Linstow, 1888, pp. 9-11); *Filaria* spp. from the "blood, peritoneal cavity, muscles of thigh and pericardium of 15 birds," including the magpie, *Gymnorhina tibicen* (Bancroft, 1889, p. 58); *Filaria* sp. from *Centropus ateralbus*, Less., and Filaria sp. from *Ninox odiosa*, Scl., from the Bismarck Archipelago (Von Linstow, 1897, p. 284).

In the present case, we have found filariae in the blood of 8 magpies *(Gymnorhina tibicen)* which died at the Melbourne

Zoological Gardens, an interval of 6 days passing between the first and second lots, and over a week between these and the last lot. They were comparatively numerous in all 8 birds, especially so in one, and were present chiefly in smears from the heart-blood, kidney and liver. They were found living and motile up to more than 45 hours after reaching us, in a liver which had been kept after reception in physiological saline. This is in marked contrast to the statement by Bancroft that "immediately after the bird is shot is the proper time to examine the blood . . if the bird is left for 6 or more hours it is difficult to find them, and after 30 hours impossible. The worms soon die and are then quickly dissolved." It is more in harmony with what Von Linstow (1891, p. 302) found in the case of the Filaria of the crow, viz., that the larvae lived for 48 hours after the death of the bird. None of our specimens were seen by us till some hours after their death.

Bancroft (1889, p. 59) assumes that the intermediate host of the filaria which he found in these fifteen species of birds, including several crows, would be the lice of the bird, since on the Blue Mountain parrot he had noted the occurrence of a blood-sucking louse. This is somewhat at variance with the opinion expressed by Von Linstow, that the crow becomes infected directly by eating the infested entrails of dead birds (Von Linstow, 1891, p. 303). No other parasites, either external or internal, could be found on these birds, several of which were most carefully examined for this purpose. The absence of adult filariae from all these specimens is, perhaps, not to be wondered at since Dr. Bancroft, after an evidently very careful search, found them in not more than 6 individuals out of 112 birds, and 3 out of "a large number."

As to the general condition of the birds, 2 were in fairly good condition, though the others were extremely thin, otherwise appearing healthy.

No indications were present as to the cause of death of the birds, other than the presence of these filarial larvae, though in the light of their presence in so many birds apparently shot, as shown by Dr. Bancroft, their pathological effect cannot be regarded as certain. As will be seen on reference to the figures, 3 types of larvae are present. Whether they represent different

stages of the same species of parasite, as one might assume, is at least questionable since the larger forms are markedly less differentiated in character than the smaller forms. That they are not due to differences in method of fixing or staining is evident from the frequency with which the two dissimilar forms are met on the same slide, and even almost alongside each other. Further, many of the smears were made from blood or organs containing living larvae. These different forms were met with in all the smears, No. 2 being much the least frequent.

No. 1.—Elongated and cylindrical, with a bluntly rounded head end, and a bluntly tapered tail. Generally 98 μ long by 4.5 μ wide, i.e., rather less than a red blood corpuscle in diameter. The head is hyaline for 5.6 μ long, showing only 2 nuclei on one surface, deeply staining blue with Giemsa, and behind this a row of large, clearer, more homogeneous nuclei faintly stained pink. The bulk of the body is occupied by a series of deeply staining (blue) nuclei which appear to form the periphery of the cellular body, and to enclose a longitudinal series of pink-stained nuclei similar to those described above, though rather smaller, presumably the future alimentary canal. At the point marked 2 in Fig. 1, these or rather larger similar nuclei form a group of cells. The embryonic sheath was not visible, but the clear colourless external body-wall can be seen sometimes to show minute transverse striations such as those described by Manson in *Filaria bancrofti*, var. *nocturna* (Manson, p. 550).

The clear spot, Manson's "V-spot," found near the head end in some other filarial larvae, does not appear to be present as such here. At the points marked (4) and (5) two small irregular "breaks" in the line of nuclei can be seen. At the point marked (1), 1-7th of the length of the body from the tail end, the well-marked "tail-spot" is visible, while at (3) a sharply defined diamond-shaped clear area is visible which does not seem to be represented in exactly the same place in other filarial larvae. It is 5 μ long.

No. 2.—This is the least frequent of the 3 types, and shows a much sharper and less hyaline head. The body generally is a very faint blue with less deeply stained, more pinkish nuclei than in No. 2. Rarely the "pink nuclei" (which are much less

distinct here) in the centre of the body, are seen to be arranged in an irregular double line, especially in the head region. The gaps corresponding to 1, 3 and 4 in No. 1 are present, but in somewhat different positions in the length of the body. In the place of the special group of nuclei marked 2 in Fig. 1, there is occasionally a clear round pink spot surrounded by a deep blue zone. This form of larva is generally 108 μ long by 5.6 μ wide.

No. 3.—The third type of larva, as shown in Fig. 3, is very much less differentiated than either of the others, and though much more frequent than No. 2, is less so than No. 1. The head is sometimes hyaline, sometimes not, the tail is much more pointed than in either of the other types. Only one clear spot is visible, presumably the tail spot (No. 3). No difference in character or arrangement can be detected in any of the nuclei, the whole of which stain much more deeply than those of Nos. 1 and 2, giving this form a distinctly darker blue colour as compared with the other larvae.

In length these vary from 112 μ to 126 μ long (very rarely to 136 μ), and are 4.5 μ wide, i.e., narrower than type No. 2. The tail spot is generally about 3-8th of the body length from the tip of the tail.

In none of these larvae could the sheath be definitely determined.

As will be seen (Von Linstow, 1891, p. 301-2), these larvae are all distinctly smaller than those observed by Von Linstow in *Filaria tricuspis*, and by Borell in the crow, but are not unlike in size those described by Ecker and Herbst in several species of crow, as larvae of *Filaria attenuata*. It is not unlikely that the present form may prove to be one or other of these species, but in the meantime the name of *Microfilaria gymnorhinae* is used for facility of record.

EXPLANATION OF PLATES XXVIII.–XXX.

All figures drawn under obj. 1/12 oil-immersion, oc. 4, with camera lucida.

PLATE XXVIII.—PROTEOSOMA BIZIURAE, n. sp.

Figs. 1 and 2—Normal erythrocytes.
3, 9 and 13 (and ? 4, 6, 11)—Female gametocytes.
7 and 12—Young plasmodia.
14 and 15—Male erythrocytes.
5—Parasite leaving erythrocyte (?)
10—Escaped parasite, with diffuse nucleus.

PLATE XXIX.—HAEMOGREGARINA MEGALOCYSTIS, n. sp.

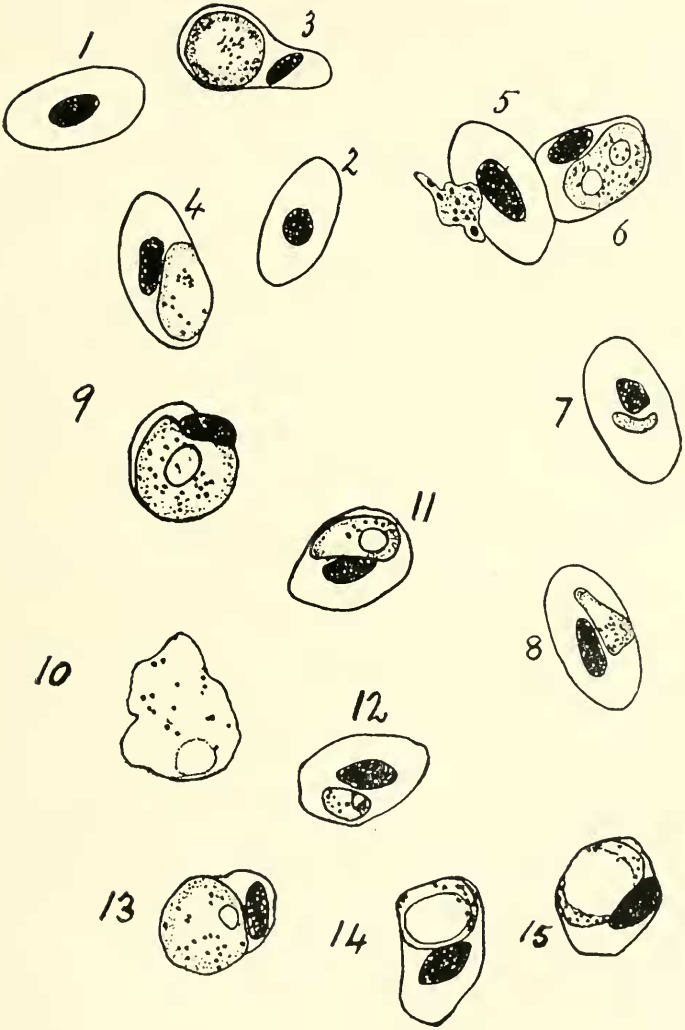
Figs. 1a to 1d—Normal erythrocytes.
2, 5, 6—Infected erythrocytes, showing degeneration of stroma but no capsule.
3, 4, 10—Infected erythrocytes, showing degeneration of stroma (ill-defined in No. 4) and capsule of parasite.
7, 11—Infected erythrocytes, without any degeneration area. Parasite not encapsuled.
11—Shows abnormally hypertrophied nucleus, but normal stroma.
8.—Leucocyte, showing chromatin rosette.
9a, 9b—Hypertrophied erythrocytes, containing no parasite.
12.—Erythrocyte, normal except in shape and more faintly staining nucleus.

PLATE XXX.—MICROFILARIA GYMNORHINAE, n. sp.

Figs. 1 to 3—Showing 3 types of larvae.

BIBLIOGRAPHY.

Bancroft, 1889.—Proc. Roy. Soc. Queensland, vol. vi., 1889.
Cleland and Johnston, 1909.—Proc. Linn. Soc. N.S. Wales, vol. xxxiv., 1909, p. 505.
Doflein.—Lehrbuch der Protozoenkunde.
Johnston, 1909.—Agric. Gaz. N.S. Wales, vol. xx., 1909, p. 581.


1
3
5
2
4
6
9
7
11
8
10
12
13
14
15